AN APPROACH TO THE MECHANISM OF MICROBIAL DECOMPOSITION OF BEECH LITTER[1,2,3]

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In the earlier papers of this series on the microbial decomposition of beech litter, studies were made first on the microbial population in the litter and chemical change involved in its decomposition (Saitô 1956, 1957). Secondly the specific features and behaviours of fungi playing prominent rôles in the decomposition were investigated (Saitô 1958).

This paper is concerned with an experimental study on the particular rôles of varied groups of fungi at different stages of decomposition and on the successive changes of fungus population in laboratory cultures with the litter.

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FUNGAL GROWTH ON POWDERED LEAVES

Three species of filamentous fungi, viz. *Absidia glauca*, *Trichoderma viride* and *Penicillium lapidosum*, and two species of hymenomycetes, viz. *Collybia* sp. and *Mycena* sp., were cultivated on powdered beech leaves singly or together, one of the filamentous fungi and one of the hymenomycetes being put together.

Powdered beech leaves were passed through a 1 mm.-mesh sieve ; 1 g. of it was placed in a 50 ml. Erlenmeyer flask (5 cm. in diameter at the bottom) and moistened with 3 ml. of distilled water. After autoclaving at 110°C. for 20 minutes, the powdered leaves were inoculated with a definite amount of mycelia and incubated at 26°C. The area of a fungal colony developed from an inoculum

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placed in the middle of the surface of leaf-powder was estimated after a certain period of time. The results are set out in Fig. 1.

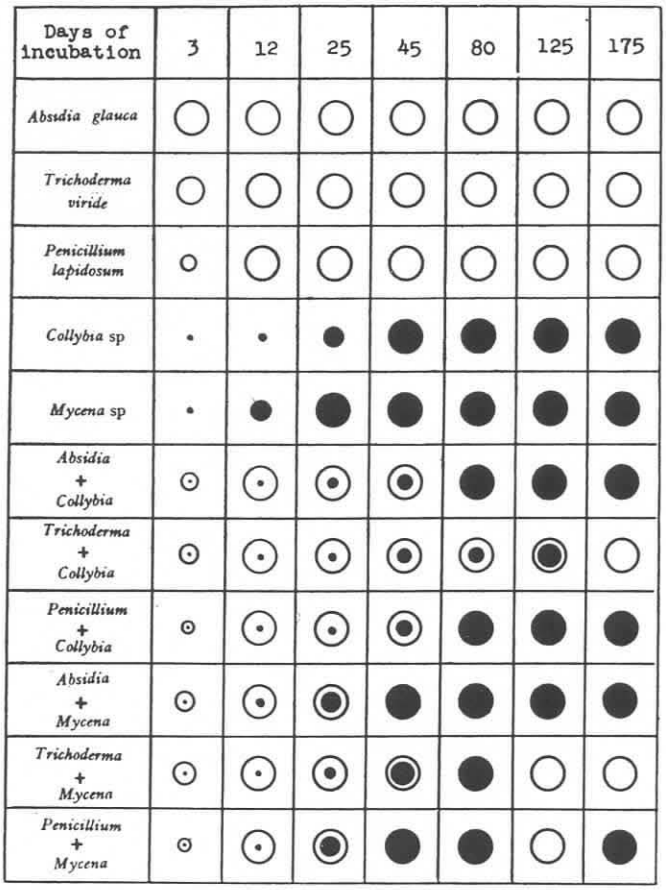

Fig. 1. The development of fungi on powdered beech leaves. Circles show relative areas of fungal colonies. White areas: the growth of a filmaentous fungus. Black areas: the growth of a hymenomycete.

When grown singly, the filamentous fungi grow rapidly, *Absidia glauca* and *Trichoderma viride*, in particular, covering the leaf-powder in three and four days respectively. However, the filamentous fungi are ephemeral, beginning to wilt after 12, 18 and 25 days in *Absidia glauca*, *Penicillium lapidosum* and *Trichoderma viride* respectively. In marked contrast, the hymenomycetes grow slowly, 18 and 45 days being necessary to cover the leaf-powder in *Mycena* sp. and *Collybia* sp. respectively.

If a filamentous fungus and a hymenomycete are grown together, an antagonistic relationship is always noticed between them, their growth being inhibited by each other. At first the filamentous fungus spreads about because of its rapid growth, but afterwards the hymenomycete becomes predominant overgrowing the former and as a result the leaf-powder is much decolorized. Nevertheless, particularly in such combinations of two fungi as *Trichoderma viride*

and *Collybia* sp., *Trichoderma viride* and *Mycena* sp., and *Penicillium lapidosum* and *Mycena* sp., the filamentous fungi grow again vigorously after a long period of the exclusive growth of the hymenomycetes. This secondary development of the filamentous fungi is rather better than their primary growth at the beginning of culture. *Absidia glauca* dies off after 150 days, when combined with the hymenomycetes.

Garrett (1956) has noticed a similar fact that phycomycete sugar fungi are able to exploit a wide variety of plant tissue ahead of competitors by virtue of their high germination and growth rate, while lignin decomposers meet with a shortage of readily available nutrients owing to their late arrival.

THE CAPACITIES OF DECOMPOSING POWDERED LEAVES OF FUNGI

The loss in weight of the infected powdered leaves was estimated with single and mixed cultures for a period of 175 days.

As will be seen from Fig. 2, the hymenomycetes, *Mycena* sp. in particular, are capable of decomposing the leaf-powder to a by far greater degree than the

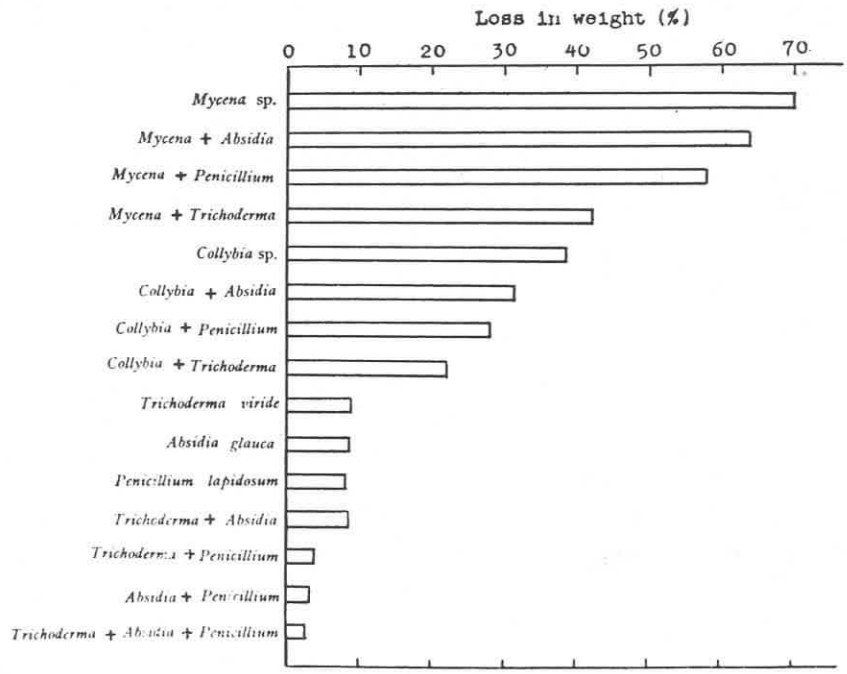

Fig. 2. The loss in weight of powdered beech leaves after 175 days' incubation.

filamentous fungi. The rate of decomposition is reduced when a hymenomycete is mixed with a filamentous fungus owing to their antagonistic relation than when infected with a hymenomycete alone. Above all the concomitant presence of *Trichoderma viride* is most inhibitory, and next comes *Penicillium lapidosum*.

CHANGES IN CONSTITUENTS AND pH IN INFECTED POWDERED LEAVES

Powdered leaves were inoculated with *Trichoderma viride* and *Collybia* sp. and incubated for 175 days, and then analysed for hot water-soluble substances, cellulose and lignin by the methods described previously (Saitô 1957). On the other hand, reducing sugar was estimated by Bertrand method in cold-water extract of powdered leaves incubated with *Trichoderma viride* for 25 days and with

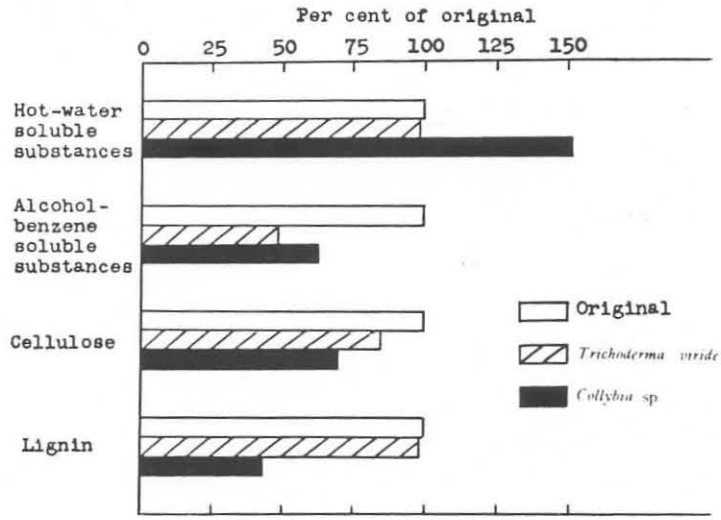

Fig. 3. Amounts of various constituents of powdered beech leaves after 175 days' incubation.

Collybia sp. for 80 days.

In what is infected with *Trichoderma viride* a slight decrease in hot water-soluble substances is observed, whereas an increase half as much as the original amount in these substances is found when infected with *Collybia* sp. Cellulose is decomposed by both fungi, more strongly by *Collybia* sp. *Collybia* sp. decomposes lignin to such a great extent as twice as much as cellulose.

Lindeberg (1944), who had studied *Marasmius* species respecting its capacity of decomposing lignin and cellulose, later (1947) came to the conclusion, investigating forty-two species of hymenomycetes growing on litter, that most species decompose lignin and cellulose to much the same extent, while some species attack much lignin but little cellulose and only one species decompose lignin exclusively.

From Fig. 4 it will be seen that in the leaf-powder infected with *Collybia* sp. there is a pronounced accumulation of reducing sugar such as four times as much as the original amount.

Thus, large amounts of the hot water-soluble substances as well as of reducing sugar, which are available as nutrients, are accumulated after the development

of the hymenomycete. This suggests that hymenomycetes create excellent nutritional conditions for the development of filamentous fungi and other

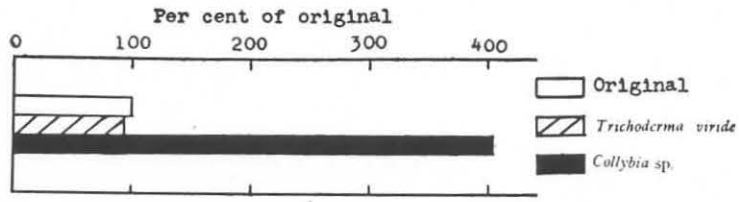

Fig. 4. Amounts of reducing sugar in powdered beech leaves after incubation with *Trichoderma viride* for 25 days and with *Collybia* sp. for 80 days.

microorganisms, and it is because of this that, in reality, the filamentous fungus associated with the hymenomycete in the same culture with the leaf-powder shows a secondary development after the hymenomycetous growth.

In this connection, Waksman and Nissen (1932) have pointed out that horse manure becomes richer in water-soluble substances and ash and poorer in lignin and cellulose than before, when infected with *Agaricus campestris*. And again, Waksman and Allen (1932) have demonstrated that horse manure can be decomposed much more readily than before after infected with this fungus owing presumably to the increase in water-soluble matter and ash.

The author (1956, 1957, 1958) has already outlined the sequence of changes in the external appearance of leaves and that in fungal populations inhabiting them on the forest-floor of beechwood. And what is observed in the present experiments with laboratory cultures accounts for the findings in the natural habitat.

Needless to say, some other factors may be more or less responsible for the sequence of development of varied fungi in the course of the decomposition of leaf material. Among them is the pH relations to be discussed here based on

Table 1

pH changes during incubation of beech leaf-powder infected with a single species of fungi

Days of incubation	*Absidia glauca*	*Trichoderma viride*	*Collybia* sp.	*Mycena* sp.
0	4.6	4.6	4.6	4.6
2	5.0	5.1	4.6	4.6
4	5.1	5.4	4.6	4.6
8	5.3	5.4	4.3	4.6
11	5.3	5.5	4.1	4.5
29	5.6	5.6	3.4	3.9
33	—	—	3.4	3.8

determinations of pH of the cultures with each of *Absidia glauca*, *Trichoderma viride*, *Collybia* sp. and *Mycena* sp. by the use of glass electrodes (Table 1).

The original powdered leaves are pH 5.1 to 5.3 and pH 4.6 before and after autoclaving respectively. The two filamentous fungi cause a rapid increase in pH, while the two hymenomycetes change pH later. Undoubtedly this is due to the different rates of development of the two groups of fungi. In the natural habitat beech litter under an active decomposition by hymenomycetous mycelia shows pH 4.0 to 4.8 in the less decomposed yellowish leaves and pH 3.7 to 4.0 in the more decomposed mouldy leaves respectively.

In nature filamentous fungi such as *Absidia*, *Mucor*, *Trichoderma* and *Penicillium* are the first fungi to grow on the brown leaves shed last autumn. Old and much decomposed leaves are mouldy (mouldy leaves) being infected with basidiomycetous mycelia. And when the basidiomycetous activities sink, there grow bacteria abundantly together with filamentous fungi, whereby basidiomycetous mycelia are finally broken down. It should be borne in mind that, in nature, the yellowish leaves, which are at a stage of decomposition prior to the mouldy ones, can be infected remarkably by filamentous fungi, and that this is sometimes met with when leaves remain yellowish without changing into the mouldy under some circumstances.

DISCUSSION

Falck (1926, 1927) divided the decomposition of woody debris into two processes, namely, destruction and corrosion. In destruction, cellulose is decomposed rapidly, while lignin is largely left intact; and in corrosion cellulose and lignin are attacked equally. The process of decomposition by *Collybia* sp. and *Mycena* sp. is similar to the corrosion as will be seen from what is described above. According to Lindeberg (1947) the value of the quotient

$$\frac{\text{decomposed lignin}}{\text{decomposed cellulose}}$$

to be determined by analysis is by no means constant. In the present study with beech litter the ratio of lignin to cellulose in leaves in the natural habitat varies from 1.14 in the original brown leaves to 0.43 in the far more decomposed fibrous leaves (Saitô 1957). And a higher rate of decomposition of lignin as compared with that of cellulose is noticed also in powdered leaves inoculated with *Collybia* sp.

Apart from the liberation of the available nutrients, the dissolution of lignin, which checks the decomposition by coating cellulose as a rule, may facilitate the utilization of the membranaceous material by microorganisms.

The process of the further decomposition of the grayish brown leaves which have got rid of basidiomycetous attack (Saitô 1956, 1957) may correspond to the destruction in the sense of Falck.

SUMMARY

1. On powdered beech leaves, filamentous fungi grow rapidly but cause only a small loss in weight. In contrast to it, hymenomycetes decompose them vigorously despite their relatively slow growth.

2. The increase in water-soluble substances and reducing sugar is recorded with the progress of decomposition of lignin and cellulose when the powdered leaves are inoculated with hymenomycetes.

3. In mixed cultures of filamentous fungi and hymenomycetes, the initial flare-up of the former is soon extinguished and then the latter becomes predominant. Finally some filamentous fungi grow again on account of the accumulation of available nutrients due to the hymenomycetous activities.

4. The foregoing sequence of changes in laboratory cultures with powdered leaves accounts for what is observed in the litter in the natural habitat.

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